

## Chromosomes of Three Species of *Dorcus* (Coleoptera, Lucanidae), with a Note on their Sex Chromosome Evolution

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**Abstract** Male germ-line chromosomes are examined in three species of stag beetles (Lucanidae), six subspecies of *Dorcus titanus*, *D. metacostatus* and *D. taurus taurus*. The diploid chromosome numbers of these *Dorcus* species are 12, 14, and 10, respectively. The sex-bivalent of the first division is of parachute-type (XYp) both in *D. titanus* and *D. metacostatus*, and neoXY in *D. taurus*.

The conventional and C-banding analyses, under the supposition that *D. titanus* is a genealogical progenitor of *D. taurus*, suggest that the karyotype of *D. taurus* ( $2n=10$ , neoXY) is formed from *D. titanus* ( $2n=12$ , XYp) by autosome-genosome fusion, by which the diploid chromosome number reduces from 12 to 10 and the synaptic mode of the sex chromosomes turns from XYp to neoXY.

**Key words:** Stag beetles, *Dorcus*, Lucanidae, chromosomes.

### Introduction

We (ABE *et al.*, 1969, 1976, 1992; KUDOH *et al.*, 1970) have hitherto studied the chromosomes of 13 species of eight genera (*Ceruchus*, *Aesalus*, *Platycerus*, *Lucanus*, *Prismognathus*, *Prosopocoilus*, *Dorcus* and *Figulus*) of the Lucanidae, paying special attention to their chromosome numbers and XY synapsis.

In this report we will describe the germ-line chromosomes of three *Dorcus* species: *D. metacostatus*, six subspecies of *D. titanus* (most of these were formerly classified as distinctive species) and the nominotypical subspecies of *D. taurus*, all of which have not yet been chromosomally examined. Also their chromosomal relationships are briefly discussed.

Table 1. Materials of three *Dorcus* species used for the chromosome observation.

Species / subspecies	Collecting date	Sex No.	Locality	2n	Type
<i>D. metacostatus</i>	26 Sep. 1974	1 ♂	Yasuno, Is. Amami Oshima, Kagoshima Pref., Japan	2n=14	XYp
<i>D. titanus platymelus</i>	2 May 1979	2 ♂	Sichuan, China	2n=12	XYp
<i>D. titanus sika</i>	19 Aug. 1995	6 ♂	Puli, Taiwan	2n=12	XYp
<i>D. titanus pilifer</i>	16 Jul. 1991	2 ♂	Miyaki-gun, Saga Pref., Japan	2n=12	XYp
	10 Feb. 2000	3 ♂	Sedaka-chô, Fukuoka Pref., Japan	2n=12	XYp
<i>D. titanus elegans</i>	25 Oct. 1972	1 ♂	Yasuno, Is. Amami Oshima, Kagoshima Pref., Japan	2n=12	XYp
<i>D. titanus daitoensis</i>	15 Mar. 1999	1 ♂	Daitoh Islands, Okinawa Pref., Japan	2n=12	XYp
<i>D. titanus titanus</i>	21 Nov. 1999	12 ♂	Cameron Highlands, Malaysia	2n=12	XYp
<i>D. taurus</i>	18 Aug. 1998	1 ♂	Layaestate, Is. Langkawi, Malaysia	2n=10	neoXY
	25 Nov. 1999	4 ♂	Cameron Highlands, Malaysia	2n=10	neoXY

\* First record from Is. Langkawi.

Materials and Methods

Adult males of three *Dorcus* species, *D. metacostatus* KIKUTA, *D. titanus* (six sub-species, see Table 1), and *D. taurus taurus* (FABRICIUS) were used in the present study. Identification and taxonomical treatment of beetles follow MIZUNUMA and NAGAI (1994). The localities and dates of their collection, and the number of individuals examined are summarized in Table 1. Of these, *D. t. taurus* was firstly recorded from Is. Langkawi.

Testes were either squashed after staining with aceto-orcein for *D. metacostatus* and *D. t. elegans*, or minced and air-dried for Giemsa staining for *D. t. pilifer*, *D. t. platymelus* and *D. t. daitoensis*. Those of the other species and subspecies were prepared according to CROZIER (1968). *Dorcus. t. taurus* and three subspecies of *D. titanus* were C-banded. The BSG method (SUMNER, 1972) was adopted for C-banding.

Results

All the specimens examined in the present study contained sufficient number of the first and second spermatocytes and spermatogonia to determine the haploid and diploid numbers (n and 2n) of the chromosomes. In each taxon the haploid and diploid numbers were determined with at least thirty metaphases.

1) *Dorcus metacostatus*

This species has a diploid chromosome number of 14 (2n=14), consisting of two submetacentric (SM) pairs of autosomes (Nos. 3 and 5), four metacentric (M) pairs (Nos. 1, 2, 4, and 6) and sex chromosomes, a metacentric X and an acrocentric (AC) Y (Fig. 1a). The first spermatocytes (MI) showed n=7 with a typical parachute-type XY synapsis (Fig. 1b). In the second division (MII, n=7) two classes of spermatocytes, an



Fig. 1. Chromosomes of *Dorcus metacostatus*: a, representative karyotype of *Dorcus metacostatus*; b, first-meiotic metaphase; c, second-meiotic metaphase with x-class; d, second-meiotic metaphase with y-class. (Thick arrow indicates X, and thin arrow Y chromosomes, respectively.)

X-class and a Y-class, were distinguished from each other (Fig. 1c & d). In the Y-class MII the Y chromosome was identified as acrocentric, whereas the X chromosome was not identified in the X-class MII due to coexistence of autosomes of similar morphology.

## 2) *Dorcus titanus*

*Conventional staining.* All the subspecies examined showed  $2n=12$ , and their karyotypes consisted of five pairs of large autosomes (SM in Nos. 1 and 4, and M in Nos. 2, 3 and 5) and small sex chromosomes, probably a submetacentric X and a dot-



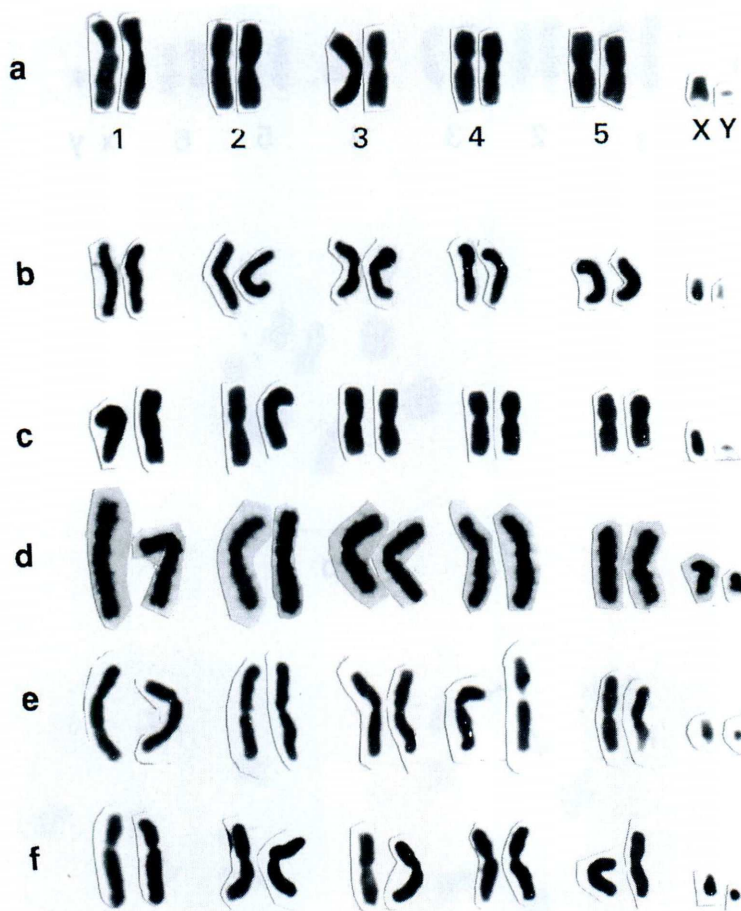
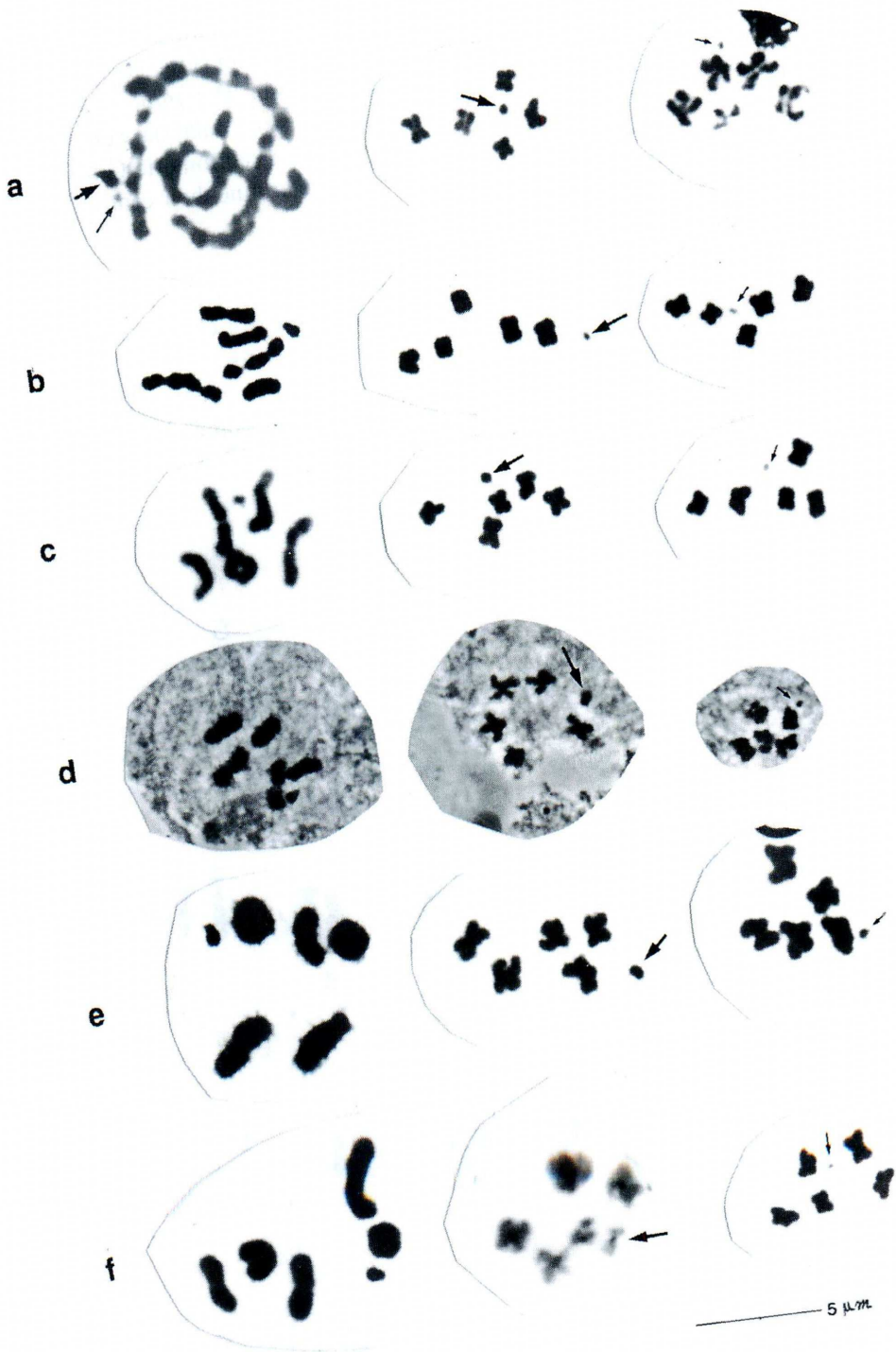


Fig. 2. Representative karyotypes of *Dorcus titanus*: a, *Dorcus titanus platymelus* from Sichuan; b, *D. t. sika* from Puli; c, *D. t. pilifer* from Miyaki-gun; d, *D. t. elegans* from Yasuno; e, *D. t. daitoensis* from the Daitoh Isls.; f, *D. t. titanus* from the Cameron Highlands.

like Y (Fig. 2).

Diplotene chromosomes of these subspecies commonly exhibited a characteristic beads-like feature irrespective of their collecting localities. A representative diplotene plate of *D. t. platymelus* from China is presented in Fig. 3a. The XY synapsis in MI

Fig. 3. First and second division of *Dorcus titanus*: a, *Dorcus titanus platymelus* from Sichuan; b, *D. t. sika* from Puli; c, *D. t. pilifer* from Miyaki-gun; d, *D. t. elegans* from Yasuno; e, *D. t. daitoensis* from the Daitoh Isls.; f, *D. t. titanus* from the Cameron Highlands. Left: First-meiotic division. Middle: Second-meiotic division, x-class. Right: Second-meiotic division, y-class. (Arrows indicate X [thick arrows] and Y [thin and long] chromosomes.)



spermatocytes was of parachute-type in this species, and thus the X-class and Y-class MII spermatocytes were easily distinguishable from each other (Fig. 3a).

*C-band staining.* *Dorcus t. sika* from Puli of Taiwan, *D. t. pilifer* from Fukuoka of Japan and *D. t. titanus* from the Cameron Highlands of Malaysia were C-banded. All the autosomes of these subspecies apparently carry a centromeric C-band, but C-bands of the sex chromosomes were too indistinct to determine their centromeric location (Fig. 4).

3) *Dorcus taurus taurus*

*Conventional Staining.* Two males from the Cameron Highlands and two males from Langkawi were examined, and no discernible variation of chromosomes was detected between these two geographically isolated populations. The karyotypes from spermatogonia consisted of three metacentric pairs and two submetacentric pairs, one of which was heteromorphic, appearing to be the sex chromosomes X and Y (Fig. 5). Thus, the diploid chromosome number was determined to be 10. The X chromosome,

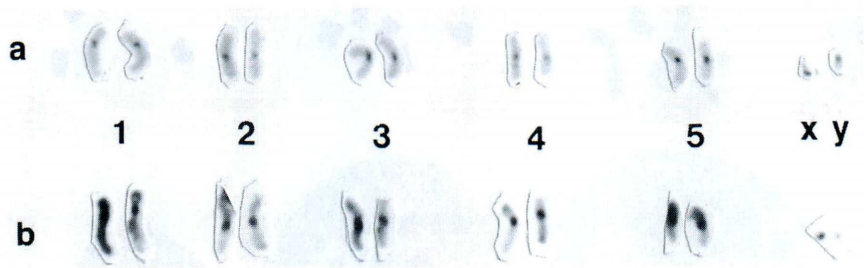


Fig. 4. C-banded karyotypes of *Dorcus titanus sika* (a) and *D. t. titanus* (b).

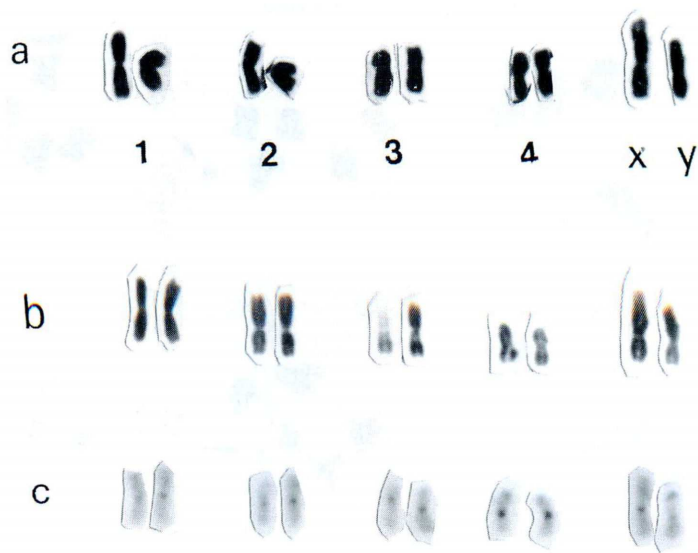


Fig. 5. *Dorcus taurus*: a, Cameron Highlands; b, Langkawi; c, Langkawi, C-banded karyotype.



Fig. 6. First and second divisions of *Dorcus taurus*, a, Langkawi. Left: First division. Right: Second division. b, Cameron Highlands. First division.

the second largest element in the complement, was quite remarkable in that the distal part of its long arm can be morphologically distinguished from the remaining part due to rather light staining. The Y chromosome was a slightly smaller SM than the X. Therefore, *D. taurus* apparently differed from both *D. metacostatus* and *D. titanus* in autosomal constitution as well as in sex chromosome morphology. The MII spermatocytes had five bivalents, of which four showed a typical dumbbell feature (so-called rod-type) and the remaining one, the largest bivalent, showed an asymmetric dumbbell feature. The latter one is supposed, judging from the spermatogonial karyotypes, to be the neoXY bivalent (neoXY) (Fig. 6 a & b).

**C-band Staining.** C-banded karyotypes were obtained only in spermatogonia from the Cameron Highlands (Fig. 5 c). In *D. taurus*, all the chromosomes including the sex chromosomes had centromeric C-bands. In addition to these centromeric C-bands, the X chromosome had a distal C-band on their long arm which was rather lightly stained by conventional staining (Fig. 5 a & b).

### Discussion

Figures 1 a and 2 a show the submetacentric chromosomes (Nos. 3, 5, and X) of *D. metacostatus* and (Nos. 1, 4 and X) of *D. titanus*, respectively. The X chromosome



of *D. metacostatus* is much larger than that of *D. titanus*. No. 1 pair of the chromosomes of *D. titanus* has longer arms than those of the other pairs, making a clear distinction in size. The number of the pairs of chromosomes in *D. titanus* is one less than that of *D. metacostatus*. If we hypothesize that *D. titanus* was diverged from a karyologically similar congener of these two species and that *D. titanus* is a phylogenetic progenitor of *D. taurus* as a result of the fusion of one of the pairs of the constituting chromosomes of *D. metacostatus* into No. 3 of itself, it will correspondingly explain the production of a large No. 1 pair of the chromosomes of *D. titanus* with the reduction of one chromosome. In spite of a distinct karyological difference between these two species, they have much in common in that the autosomes of both species are only metacentric and submetacentric, and that XY synapses are of XY parachute-type. In order to clarify rearrangements in chromosomes, further genealogical research and analyses are needed in the near future using efficient G-banding techniques.

Since *D. taurus* has one less pair of chromosomes than *D. titanus* and lacks a submetacentric pair corresponding to pair No. 1 of *D. titanus* (Fig. 5 a & b), it may be supposed, based on the chromosome morphology and C-band position, that pairs Nos. 1 and 2 of *D. taurus* correspond to pairs Nos. 2 and 3 of *D. titanus*, respectively, and in the same way pairs Nos. 3 and 4 in the former correspond to pairs Nos. 4 and 5 in the latter, respectively. If this is the case, it would be probable that the large neoXY chromosomes of *D. taurus* might have been formed by the fusion of pair No. 1 and the small XY of *D. titanus*. Taking this view into consideration, the distal C-bands of the XY chromosomes in *D. taurus* may have their origin in the sex chromosomal C-bands of *D. titanus*. So far as these two species are concerned, it is most likely that the synaptic mode of the sex chromosomes has turned from the parachute-type (XYp) to the neoXY through rearrangement by the fusion of one autosomal pair with the small XY chromosomes, and such rearrangement resulted in a marked increase in the size of the sex-chromosomes. This fashion of the chromosome rearrangement was found in *Oileus*, Passalidae by VIRKKI and REYES-CASTILLO (1972) and in *Dendroctonus*, Scolytidae by SMITH and VIRKKI (1978). However, more direct proofs might be obtained by using G-banding techniques.

In this study we have discerned no karyological differences among the six subspecies of *D. titanus*.

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## 要 約

阿部 東・工藤貢次：クワガタ属3種の染色体と核型進化。—— スジブトヒラタクワガタ、ヒラタクワガタの6亜種とタウルスヒラタクワガタの染色体を調査した。スジブトヒラタクワガタは2n, 14, XYp（パラシュート型）である。ヒラタクワガタは2n, 12, XYpで小型サブメタセントリックのX、とくに小さい点状のYからなる。各亜種間で核型の差は認められなかった。タウルスヒラタクワガタは2n, 10, ネオXYである。性染色体は2番目に大きいサブメタセントリックである。

Cバンド染色はヒラタクワガタの3亜種とタウルスヒラタクワガタで行なわれ、ヒラタクワガタでは、6対の常染色体の動原体部分にCヘテロクロマチンが確認され、小さいXでは位置がはっきりしない。タウルスヒラタクワガタでは5対の動原体性のヘテロクロマチンが認められるが、X染色体はこのほかに、長腕の先端にCヘテロクロマチンをもう1個多く有する。タウルスヒラタクワガタにおける、大型で先端にもう1個のヘテロクロマチンを有する性染色体について、もしヒラタクワガタの核型がタウルスヒラタクワガタの核型よりも祖先型を示すものと仮定するならば、タウルスヒラタクワガタの大型のX、Yは、常染色体に小型のXYが転座融合したものと考えることができる。その結果、ヒラタクワガタのように2n, 12より2個減少し、タウルスヒラタクワガタのように2n, 10、となり、タウルスヒラタクワガタの大型のXが生じ、セントメリックのCバンドのほかに、もともとのX染色体のCヘテロクロマチンが付加した（その逆もありうる）と考えることができ、XYpからneoXYに進化したと考えられよう。しかしこれは、染色体の形態からの仮説であって、Gバンド染色などによる染色体再編成の機構の調査や系統に関するさらなる研究が必要である。

## References

- ABE, A., K. KUDOH, T. ICHIKAWA & K. SAITOH, 1992. Chromosome studies of beetles. IX. Some karyological aspects of five taxa of stag beetles (Lucanidae). *Sci. Rept. Hirosaki Univ.*, **39**: 31–36.
- , I. KONDOH & K. SAITOH, 1969. Chromosome study in five species of Lucanidae (Insecta: Coleoptera). *Kontyû, Tokyo*, **37**: 179–186. (In Japanese, with English résumé.)
- , & K. SAITOH, 1976. Chromosome studies of beetles. VIII. A revised and supplemental chromosome study in the genera *Prosopocoilus*, *Nipponodorcus* and *Macrodercas* of the Lucanidae. *Sci. Rept. Hirosaki Univ.*, **23**: 50–56.
- , & ——— 1992. Male somatic chromosomes of *Platycerus delicatulus delicatulus* LEWIS, 1883 (Coleoptera, Lucanidae). *Elytra, Tokyo*, **20**: 222. (In Japanese, with English title.)
- CROZIER, R. H., 1968. An acetic acid dissociation, air-drying technique for insect chromosomes, with aceto-lactic orcein staining. *Stain Technol.*, **43**: 171–173.
- KUDOH, K., A. ABE, I. KONDOH, N. SATOH & K. SAITOH 1970. Some cytological aspects of three species of beetles. *Kontyû, Tokyo*, **38**: 232–238.
- MIZUNUMA, T., & S. NAGAI, 1994. The Lucanid Beetles of the World. 337 pp.+156 pls. Mushisha, Tokyo.
- SMITH, G., & N. VIRKKI, 1978. Animal Cytogenetics 3 Insecta 5: Coleoptera. Gebruder Borntraeger, Berlin / Stuttgart.
- SUMNER, A. T., 1972. A simple technique for demonstrating centromeric heterochromatin. *Exptl. Cell Res.*, **75**: 304–305.
- VIRKKI, N., & P. REYES-CASTILLO, 1972. Cytotaxonomy of Passalidae (Coleoptera). *Anais Esc. nac. Cienc. biol., Méx.*, **19**: 49–83